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## Post-Meiotic DNA Damage and Response in Male Germ Cells

Frédéric Leduc et al.\*

*Department of Biochemistry, Faculty of Medicine and Health Sciences  
Université de Sherbrooke  
Canada*

### 1. Introduction

Spermatids are haploid cells that differentiate into spermatozoa and may be considered as an interesting model of DNA damage response and repair. Key features, such as a unique set of chromosomes, radioresistance to apoptosis, the presence of known end-joining DNA repair pathways and an underlying prerogative to limit the transmission of any mutation to the next generation, make them a unique cell type to provide new insights on similar pathways in somatic cells. Although DNA damage signaling and repair mechanisms have been extensively studied during meiosis, the contribution of post-meiotic germ cells to the genetic integrity of the male gamete have been overlooked. In this chapter we present clear evidences that the haploid phase of spermatogenesis, termed spermiogenesis, may represent an even greater challenge for the maintenance of the genetic integrity of the male gamete. Since transient DNA strand breaks are intrinsic to the differentiation program of spermatids (Leduc et al., 2008a; Marcon and Boissonneault, 2004), a better understanding of DNA repair pathways involved may shed some light on their potential contribution to male-driven *de novo* mutations and eventually to some unresolved cases of male infertility. This chapter will mainly focus on DNA breaks occurring in the post-meiotic phase of the spermatogenesis and how germ cells deal with it.

### 2. Spermatogenesis

In most mammals, testes are found in the scrotum and are maintained at lower temperature (2-8°C) than the core body (Harrison and Weiner, 1949; Setchell, 1998). In fact, spermatogenesis is known to work better at lower temperature and it was shown that fertility declines with scrotal hyperthermia. For example, higher scrotal temperature due to fever or lifestyle correlates with decreased semen quality in humans (reviewed in Jung and Schuppe, 2007).

To support germ cells in their development, Sertoli cells are located at the basal lamina, throughout the seminiferous tubules (Russell, 1990). They provide nutrients and essential

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\* Geneviève Acteau, Marie-Chantal Grégoire, Olivier Simard, Jessica Leroux, Audrey Carrier-Leclerc, Mélina Arguin and Guylain Boissonneault

*Department of biochemistry, Faculty of medicine and health sciences, Université de Sherbrooke, Canada*

molecules to the differentiating germ cells and regulate the seminiferous tubular fluid (Griswold, 1998; Rato et al., 2010). The Sertoli cells are interconnected by different junctions, creating a unique barrier between surrounding blood vessels and differentiating germ cells that is known as the “blood-testis barrier” (Cheng and Mruk, 2002; Dym and Fawcett, 1970; Setchell, 1969; Vogl et al., 2008). This barrier restricts molecules to enter or exit the adluminal compartment, creating a microenvironment with diverse transporters and preventing immunological response against germ cells (reviewed in Mital et al., 2011).

Spermatogenesis is the cellular differentiation pathway leading to the production of male gametes. This process takes place in seminiferous tubules in the testis, which is a unique environment regulated by follicle-stimulating and luteinizing hormones, secreted by the pituitary gland (Russell, 1990). From birth to puberty, seminiferous tubules are composed of spermatogonia, the precursor stem cell of the germinal cells, and Sertoli cells. At the onset of puberty, spermatogonia undergo mitosis and commit to the differentiation pathway leading to male germ cells. As the germ cells differentiate, they migrate towards the lumen of the tubule, creating an organized stratified structure. Spermatogenesis can be divided in two phases, spermatocytogenesis and spermiogenesis.

Spermatocytogenesis is the process by which a spermatogonium differentiates into primary spermatocytes, which duplicate their DNA to undergo meiosis and become haploid spermatids. This meiotic division is important to create genetic variations by meiotic crossovers and random segregation of parental chromosomes. Spermiogenesis is characterized by the radical metamorphosis of the haploid spermatid into spermatozoa, requiring the reorganization of their organelles. The acrosome, a cap-like structure needed for enzymatic digestion of the oocyte outer membrane, is formed from the Golgi apparatus. At the opposite nuclear pole, the flagellum begins to grow from the centrioles and mitochondria groups at the mid-piece of the emerging flagellum to produce the required energy for its later motion. Finally, the spermatid is stripped of most of its cytoplasm and ultimately released in the lumen of the seminiferous tubule. Most interestingly, the nucleus of spermatids is also remodeled and condensed to protect the paternal genome as well as providing a more hydrodynamic shape. However, this nuclear reorganization is characterized by transient DNA strand breaks that may be necessary to relieve the torsional stress as outlined below.

## 2.1 Chromatin remodeling process

Through the chromatin remodeling process of spermatids, the paternal genome is condensed tenfold compared to somatic cells, forming a nucleus with an hydrodynamic-shape (Balhorn et al., 1984). To achieve such a high degree of compaction, chromatin must first rely on a set of abundant transition proteins (TPs) subsequently replaced by the protamines (PRMs) (Balhorn et al., 1984; Braun, 2001). The arginine-rich PRMs bind DNA and neutralize the phosphodiester backbone of the double helix (Balhorn, 1982), allowing for a tight compaction of the DNA into torroids (Ward, 1993). Although the onset of chromatin remodeling is poorly understood, incorporation of testis-specific histone variants (Churikov et al., 2004; Govin et al., 2007; Lu et al., 2009; Martianov et al., 2005; Yan et al., 2003) and regulated post-translational modifications of histones, such as acetylation (Christensen et al., 1984; Grimes and Henderson, 1984; Marcon and Boissonneault, 2004; Meistrich et al., 1992), ubiquitination (Baarends et al., 1999; Chen et al., 1998), methylation (Godmann et al., 2007; van der Heijden et al., 2006) and phosphorylation (Blanco-Rodríguez,

2009; Krishnamoorthy et al., 2006; Leduc et al., 2008a; Meyer-Ficca et al., 2005) are known to initiate and participate in the exchange from histones to the more basic proteins such as TPs and PRMs during the transition from the round to the elongated spermatids. These modifications are known to modulate the affinity of histones for DNA, but also the affinity of other proteins for histones, such as chromatin remodelers, DNA repair proteins or the transcription machinery. After spermiation, this unique protamine-based chromatin is further stabilized by the creation of intraprotamine disulfide bonds during the transit through the epididymis (Golan et al., 1996). Therefore, protamination provides both chemical and mechanical protection to the male haploid genome. Interestingly, protamination of the male genome is not complete and varies across species. In the mouse spermatozoon, about 1-2% of the genome remains organized by histones (Balhorn et al., 1977), whereas up to 15% of histones are still found in humans spermatozoa (Gatewood et al., 1990; Gusse et al., 1986; Tanphaichitr et al., 1978). This observation lead to hypothesize that these nucleosomes could serve as epigenetic markers for embryonic development (Arpanahi et al., 2009; Zalenskaya et al., 2000) (for a more detailed review on the sperm chromatin organization, see Johnson et al., 2011)

### **3. Nature of endogenous DNA damages during spermiogenesis**

#### **3.1 Single strand damage and repair**

Depending on the type of damage, specific pathways achieve single strand damage repair (see Table 1). Mismatched DNA bases that primarily arise during replication are corrected by mismatch repair (MMR), while small chemical alterations of DNA bases such as alkylation, deamination and oxidative damage are repaired by base excision repair (BER) (Mukherjee et al., 2010; Robertson et al., 2009). More complex lesions such as those induced by UV (pyrimidine dimers and helix-distorting lesions) are corrected by nucleotide excision repair (NER), a multistep pathway that involves more than 30 proteins (Hoeijmakers, 2009; Nospikel, 2009). DNA nicks are repaired by single-strand break repair (SSBR). These DNA repair pathways are known to be present and active during spermiogenesis (Olsen et al., 2001; Schultz et al., 2003). To our knowledge, single-strand damages do not present a major threat to spermatids. With the exception of exposures to toxicant that could challenge these pathways, in normal conditions, single-strand DNA damage during spermiogenesis is likely attributed to the massive transcription that is taking place at these steps and is efficiently resolved by spermatids (Olsen et al., 2001). DNA double-strand breaks were reported as part of the normal differentiation program of spermatids during spermiogenesis which may represent an important source of genetic instability and therefore we will focus on these pathways.

#### **3.2 Double-strand breaks in spermatids**

##### **3.2.1 Possible origin of DNA breaks**

Several hypotheses have been formulated to elucidate the origin and role of DNA strand breaks in spermatids. Sakkas and colleagues suggested that “abortive apoptosis” may be the cause since abnormal human spermatozoa presented some apoptotic-like features (Sakkas et al., 1999). Further investigation led to the demonstration that other biomarkers of apoptosis in sperm cells were present such as BCL-X, TP53, caspases, in addition to diverse structural defects (Baccetti et al., 1997; Donnelly et al., 2000; Gandini et al., 2000; Sakkas et al., 2002; Weng et al., 2002). Due to technical limitations at the time, DNA breaks were only observed

DNA repair pathways		DNA damages	Implicated proteins
Mismatch repair (MMR)		Mispaired DNA bases	MSH1-6, MLH1, MLH3, PMS1, PMS2, EXO1, RPA, PCNA, RFC
Base excision repair (BER)	Short-patch	Small DNA bases chemical alteration arising from alkylation, deamination and oxidative damage	UNG, APEX1, POL $\beta$ , XRCC1, LIG3
	Long-patch		UNG, APEX1, POL $\beta$ / $\delta$ , FEN1, PCNA, LIG1
Nucleotide excision repair (NER)	Transcription-coupled or not	Pyrimidine dimer	XPC complex, DDB complex, ERCC3 (TFIIH), XPA-RPA complex, ERCC5 (XPG), ERCC1-ERCC4 (XPF), LIG3, DNA polymerase $\delta$
Single strand break repair (SSBR)	Short-patch or long-patch	Single strand break (SSB)	APE1, PNKP, APTX, TDP1, POL $\beta$ / $\delta$ / $\epsilon$ , PCNA, XRCC1, LIG1/3, FEN1, PARP

Table 1. Summary of the single strand DNA repair pathways in mammalian cells (Ciccia and Elledge, 2010; Hoeijmakers, 2009; Martin et al., 2010; Mukherjee et al., 2010; Nouspikel, 2009; Robertson et al., 2009).

in a subset of the whole population of elongating spermatids and therefore abortive apoptosis could represent a sound explanation. However, some studies demonstrated that round spermatids are radioresistant to apoptosis and may not have the proper machinery and checkpoints to trigger such process (Ahmed et al., 2010; Oakberg and Diminno, 1960). Furthermore, our group have demonstrated that transient DNA breaks were present in the whole population of elongating spermatids of fertile mice and humans during chromatin remodeling and were therefore part of the normal differentiation program of these cells (Marcon and Boissonneault, 2004). The persistence of these breaks beyond the chromatin remodeling steps in pathological conditions may explain the presence of DNA fragmentation found in spermatozoa of infertile men (Leduc et al., 2008b).

Generation of controlled DNA breaks either single- or double-stranded may be important to relieve the torsional stress induced by the withdrawal of histones (Boissonneault, 2002). The simple mechanical stress resulting from the accumulation of free supercoils could induce non-B DNA structures and possibly DNA breaks as the chromatin remodeling is extensive and takes place within many differentiation steps. However, enzymatic induction of DNA strand breaks is more likely, as their free ends can be end-labeled with polymerases that require a 3'OH as substrate, such as the terminal deoxynucleotidyl transferase (TdT) used in TUNEL labeling. Specific nucleases could be involved in this process, and it is not excluded that retrotransposon nucleases could play a role as they are expressed throughout

spermatogenesis, including in the nucleus of spermatids (Branciforte and Martin, 1994; Ergün et al., 2004; Gasior et al., 2006). However, topoisomerases have long been considered likely candidates to support chromatin remodeling from bulky histone-bound chromatin to compact and transcriptionally inert protamine-bound DNA because of their ubiquitous role in chromosome dynamics during the somatic cell cycle (McPherson and Longo, 1993).

### 3.2.2 Topoisomerases as candidates to supercoiling removal

Change in DNA topology can be achieved by single-strand breaks (SSBs) generated by type I topoisomerase, which modifies the linking number in steps of one. Single-strand breaks would be considered a much smaller threat for the genome's integrity of spermatids than a DSB that could be generated by type II topoisomerases. However, chromatin remodeling in spermatids was clearly shown to be associated with an increase in type II topoisomerase (Chen and Longo, 1996; Laberge and Boissonneault, 2005; Leduc et al., 2008a; McPherson and Longo, 1992, 1993; Meyer-Ficca et al., 2011b; Roca and Mezquita, 1989). A possible link between type II topoisomerases and DNA breaks found in elongating spermatids was suggested by the elimination of DNA breaks in spermatids nuclei incubated with type II topoisomerase inhibitors such as suramin and etoposide (Laberge and Boissonneault, 2005). In mammal cells, the  $\alpha$  and  $\beta$  isoforms of topoisomerase share more than 80% of homology and are differentially expressed. Topoisomerase II $\alpha$  (TOP2A) is mostly found in replicating cells whereas topoisomerase II $\beta$  (TOP2B) predominates in quiescent cells (Morse-Gaudio and Risley, 1994; Turley et al., 1997). Using immunofluorescence on mouse testis sections, we have observed TOP2B foci in nuclei of elongating spermatids whereas TOP2A remained undetected in these cells but highly present in spermatocytes (see Figure 1) (Leduc et al., 2008a). Detection of TOP2B in elongating spermatids is not surprising, as spermatids are non-replicative cells. Recent studies confirmed the involvement of TOP2B in elongating spermatids (Meyer-Ficca et al., 2011b) and also observed its presence further downstream of the male germ cells differentiation program as part of the nuclear matrix of sperm cells, supporting its earlier role in the chromatin remodeling of spermatids (Shaman et al., 2006).

### 3.2.3 Topoisomerases and DNA repair

Type II topoisomerase activity may be modulated by post-translational modifications, such as phosphorylation by kinases and poly (ADP-ribosyl)ation by poly (ADP-ribose) polymerases (PARPs), a well-known family of proteins involved in a multitude of nuclear events, such as DNA repair and chromatin remodeling. This complementary interaction between TOP2B and PARPs may be involved in numerous cellular processes. For example, TOP2B and PARP1 are known to modulate transcription in somatic cells (Ju et al., 2006). Furthermore, these proteins may be important constituents of the nuclear matrix; Zaalishvili and coworkers observed the stimulation of cleavage of nuclear matrix associated DNA loops of neuron and leukocyte nucleoids when incubated in buffer supporting topoisomerase and PARP activity (Zaalishvili et al., 2005). This stimulation was reversed by the addition of thymidine, a PARP inhibitor. The authors suggested that a PARP-modified topoisomerase II may cut efficiently but the (ADP-ribosyl)ation could inhibit the religation. Recently, Meyer-Ficca and colleagues demonstrated a possible modulation of TOP2B activity by PARP and PARG *in vitro* using recombinant proteins as well as *in vivo* during mouse spermiogenesis through the use of inhibitors and knockout mouse models (Meyer-Ficca et al., 2011b). According to their findings, there is a functional relationship between the DNA strand break activity of TOP2B and the DNA strand break-dependent activation of

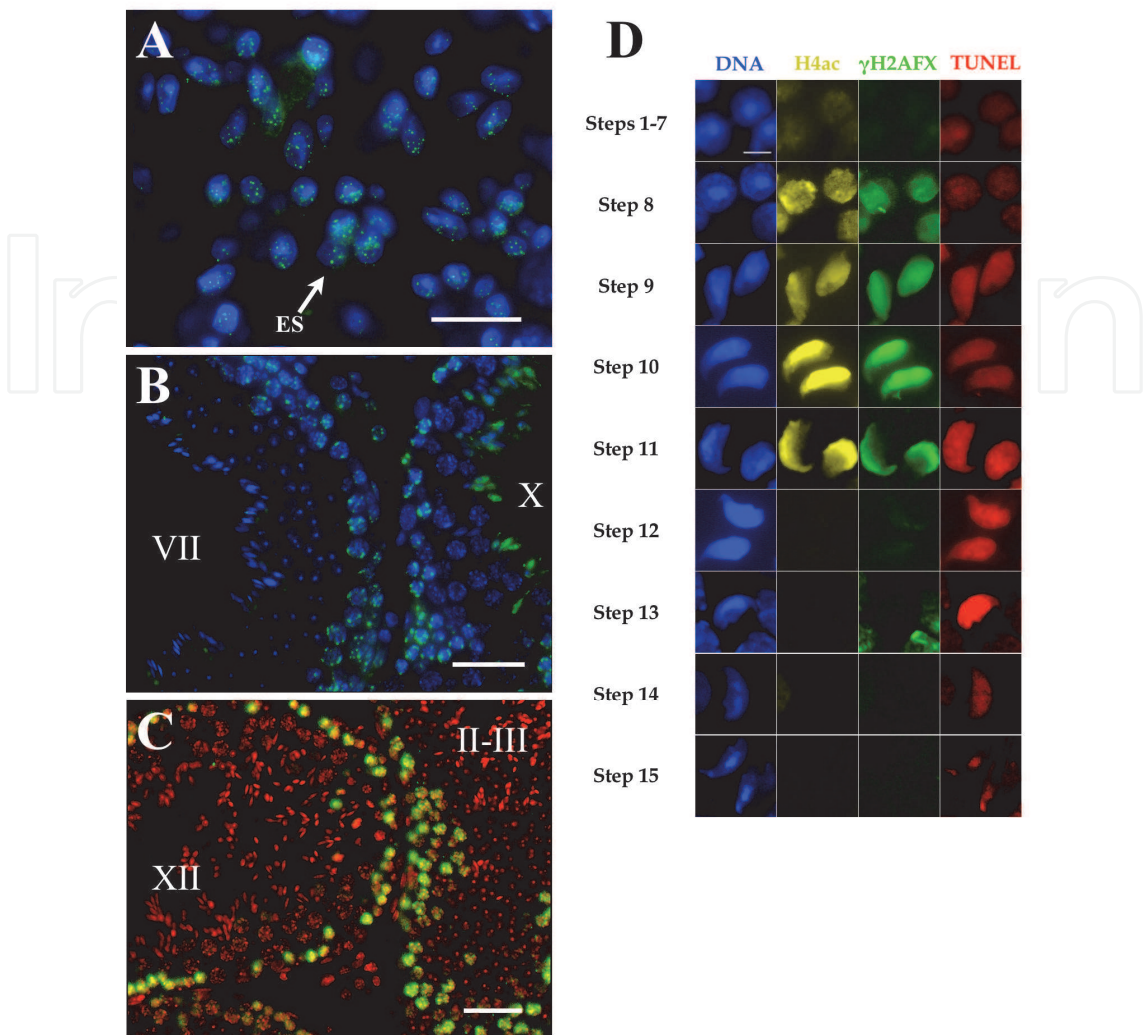


Fig. 1. Presence of type II topoisomerases, hyperacetylated histone H4,  $\gamma$ H2AFX and DNA breaks during mouse spermiogenesis. (A) Overlay of TOP2B immunofluorescence (green) and DAPI nuclear staining (blue) of a stage IX tubule demonstrating the presence of TOP2B in nuclei of elongating spermatids (ES) at the onset of chromatin remodeling. (B) Overlay of TOP2B immunofluorescence (green) and DAPI (blue) nuclear staining of stages VII and X tubules. (C) Overlay of immunofluorescence of TOP2A (green) and TO-PRO3 (red) nuclear staining of stages XII and II-III demonstrating the nuclear presence of TOP2A in zygotene and pachytene spermatocytes but complete absence in spermatids. (D) Detection of hyperacetylated histone H4 and  $\gamma$ H2AFX by immunofluorescence and DNA breaks by TUNEL during mouse spermiogenesis. DNA was counterstained by TO-PRO3. (A-C) Immunofluorescence on Bouin-fixed testis sections was done as previously described (Leduc et al., 2008a). (D) Squash preparation were done as previously described (Kotaja et al., 2004; Leduc et al., 2008a), fixed with ice-cold ethanol and processed for TUNEL and immunofluorescence. Bars = 10  $\mu$ m (A and B), 20  $\mu$ m (C) and 5 $\mu$ m (D).

PARP enzymes. Moreover, alteration in the PAR metabolism leads to a greater retention of histones in spermatozoa (Meyer-Ficca et al., 2011a). Whether PARP1 is involved directly in chromatin remodeling, DNA repair or combination of both in spermatids remains to be determined and will be discussed further in section 4.

## 4. DNA damage response and DNA repair of double-strand breaks

### 4.1 DNA damage signaling pathways

The first step following a DSB is the detection of the DNA damage by sensors (Lamarche et al., 2010). At least four independent sensors can detect DSBs: PARPs in all cases of SSBs and, to a lesser extent, DSBs, Ku70/80 in D-NHEJ, MRE11-RAD50-NBS1 (MRN) complex in all cases of DSBs and replication protein A1 (RPA) in HR (Ciccia and Elledge, 2010; Lamarche et al., 2010).

As previously stated in section 3.2.3, the presence and activity of PARP1 and PARP2 have been recently investigated during spermiogenesis of mouse and rat (Ahmed et al., 2010; Dantzer et al., 2006; Meyer-Ficca et al., 2005; Meyer-Ficca et al., 2011a; Meyer-Ficca et al., 2009; Meyer-Ficca et al., 2011b). Although the individual absence of these proteins leads only to subfertility in male, it is believed that they play a key role in the maintenance of genomic integrity of spermatids. As discussed previously, PARPs may be involved in DNA repair and signaling, in the drastic chromatin remodeling of spermatids and even in the repackaging of their genome with protamines (Qu  net et al., 2009). However, the embryonic lethal phenotype of double knockout mouse prevent a better assessment of their critical role during spermiogenesis, as the absence of one can be compensated for by the other. The Ku heterodimer binds to DSB ends and is required for the non-homologous end-joining pathway (NHEJ). In addition to its role in DNA repair, Ku proteins are also required for the maintenance of telomeres and subtelomeric gene silencing (Celli et al., 2006; Lamarche et al., 2010). KU70 is present during the spermiogenesis of mouse (Goedecke et al., 1999; Hamer et al., 2003), human (Leduc *et al.*, unpublished observations), and grasshoppers (Cabrero et al., 2007), but seems to decrease as spermiogenesis proceeds, most notably after the expulsion of histones. Although initial analyses of the implication of MRN complex as sensor in non-homologous end-joining pathways produced conflicting results (Di Virgilio and Gautier, 2005; Huang and Dynan, 2002), recent studies showed that siRNA mediated knockdown of Mre11 results in reduced end-joining efficiency in both D-NHEJ and B-NHEJ pathways (Rass et al., 2009; Xie et al., 2009) and should be considered a good candidate for DNA breaks detection and signaling in spermatids. As for Ku proteins, Mre11 is also present during spermiogenesis (Goedecke et al., 1999). Contrary to these DNA break sensor proteins, RPA may not play such an important role during spermiogenesis as spermatids, being haploid, cannot rely on HR repair processes.

The detection of DNA damage by sensors activates proteins of the phosphatidylinositol 3-kinase-like protein kinase (PIKKs) family such as ATM, ATR, and DNA PKcs and members of the PARP family. These proteins post-translationally modify key protein targets triggering a signal transduction cascades that forms the DNA damage response (DDR) (Lamarche et al., 2010). During mouse spermiogenesis, ATM and DNA PKcs are present and active (Ahmed et al., 2010; Scherthan et al., 2000). These kinases are responsible for the phosphorylation of the histone H2A variant, H2AFX, at serine 139 ( $\gamma$ H2AFX, previously referred to as  $\gamma$ H2AX), which quickly occurs after a DSB. This modification can spread up to a 2 Mbp region flanking all DSBs (Kinner et al., 2008) and it could help the recruitment of other proteins of the DDR (Celeste et al., 2003). Within minutes following DNA damage,  $\gamma$ H2AFX appears at discrete nuclear foci that dissolve after the completion of DNA repair. It remains unclear whether  $\gamma$ H2AFX is replaced completely with new H2AFX histones, or simply dephosphorylated, but strong evidences suggest that the latter mechanism is

prominent (Chowdhury et al., 2005; Rogakou et al., 1999). Therefore, the implication of  $\gamma$ H2AFX in all cases of DSBs makes it a novel biomarker for DSBs detection by immunofluorescence (Mah et al., 2010; Mah et al., 2011). Upon  $\gamma$ H2AFX signaling, specific pathways are recruited according to cell type or the cell cycle phase (Shrivastav et al., 2008). The presence of  $\gamma$ H2AFX during spermiogenesis has been first shown in rats (Meyer-Ficca et al., 2005) and we confirmed its presence at the corresponding steps during mouse spermiogenesis (Leduc et al., 2008a) (see Figure 1). As shown in Figure 1, the presence of  $\gamma$ H2AFX and hyperacetylated histone H4, a biomarker of chromatin remodeling coincides with the presence of TOP2B. These results confirm the previously published strong TUNEL labeling of elongating spermatids during chromatin remodeling (Laberge and Boissonneault, 2005; Marcon and Boissonneault, 2004). Therefore, spermatids undergo multiple transient DSBs, inducing a classic DDR signaling. In addition, as seen by immunofluorescence in Figure 1,  $\gamma$ H2AFX is present in all spermatids throughout chromatin remodeling as part of the normal process of maturation of spermatids. The pattern of  $\gamma$ H2AFX in spermatids as seen in Figures 1 and 2 is dependent on fixation and tissue processing; ethanol fixation provides a better context for TUNEL labeling but alters nuclear distribution of proteins. Furthermore, we have also found the presence of  $\gamma$ H2AFX and DNA breaks during human spermiogenesis (see Figure 2), while other groups subsequently demonstrated similar DDR signaling in grasshoppers (Cabrero et al., 2007) and even in the algae *Charas vulgaris* (Wojtczak et al., 2008). Moreover, the presence of DNA breaks has also been found during spermiogenesis of drosophila (Rathke et al., 2007). Altogether, these results suggest that the DDR triggered by endogenous breaks in spermatids is evolutionary conserved and could represent a new source of male-driven genetic instability in species where gametogenesis requires condensation of the genetic material.

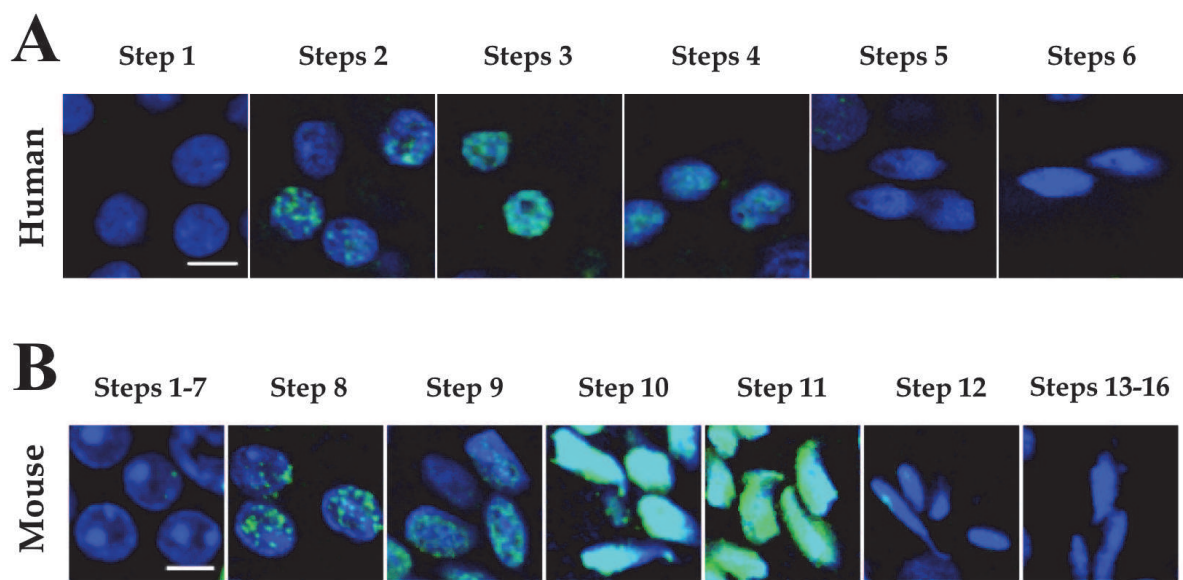


Fig. 2. Detection of  $\gamma$ H2AFX during spermiogenesis of human (upper panel), and mouse (lower panel). DNA was counterstained by TO-PRO3. Bars = 5 $\mu$ m. Immunofluorescence on paraformaldehyde-fixed testis sections was done as previously described (Leduc et al., 2008a).

#### 4.2 Evidences of an active DNA repair system during spermiogenesis

Although these DSBs are considered the most harmful genetic damage for a cell, we know from experimental data (Marcon and Boissonneault, 2004) that these breaks are repaired by the end of spermiogenesis in fertile animals. The disappearance of  $\gamma$ H2AFX in mouse spermatids (step 13 to 16) shown in Figure 1 cannot be associated with completion of DNA repair or dephosphorylation as a majority of histones are expelled from the nucleus to be replaced by PRMs. However, we obtained other evidences of an active DNA repair system at these steps by demonstrating incorporation of dNTPs *in situ* that is sustained through all the chromatin remodeling steps (see Figure 3) (Leduc et al., 2008a). Furthermore, as seen in Figure 1, the appearance and disappearance of TUNEL labeling is coincident with  $\gamma$ H2AFX fluorescence. To confirm that the loss of TUNEL labeling was associated with DNA repair and not with the lack of penetrability of the TdT in the nuclei of condensed spermatids, we decondensed spermatids prior to TUNEL with similar results (Marcon and Boissonneault, 2004) (Acteau et al., unpublished observations). Therefore, DNA breaks are properly repaired by the end of the spermatids differentiation program. As previously stated, mammalian cells can rely on four DNA DSBs repair pathways, each of which having different degree of fidelity. As spermatids differentiate to spermatozoa with fertilizing potential, any errors due to faulty or incomplete DNA repair may be transmitted to the next generation. Severe alteration in the repair process may cause infertility or possibly be incompatible with embryonic development (Leduc et al., 2008b).

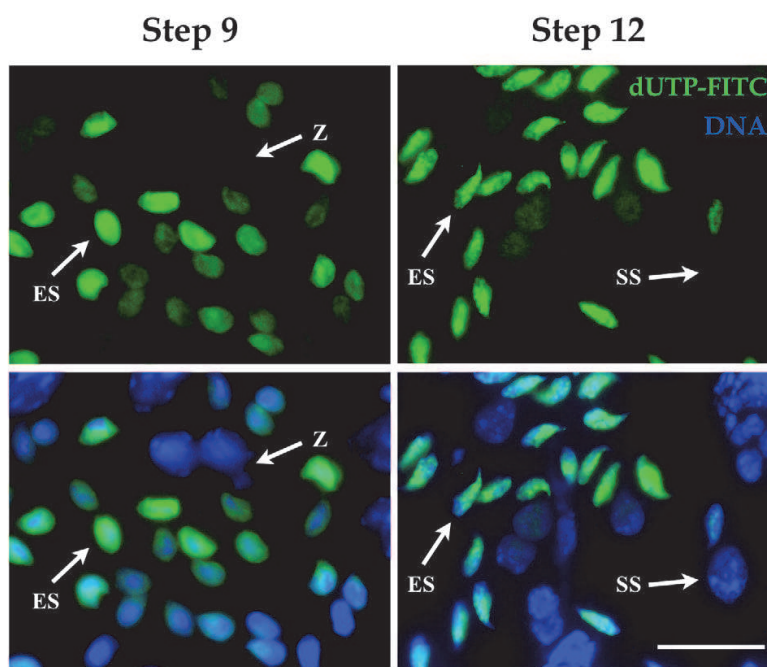


Fig. 3. In situ endogenous DNA polymerase activity assay (Leduc et al., 2008a) on squash preparation of stage IX and XII tubules. DNA was counterstained by DAPI. Bar = 10  $\mu$ m

#### 4.3 Towards identification of DNA repair pathways

Double-strand breaks are processed either by homologous recombination, single-strand annealing (SSA) or non-homologous end-joining (Caldecott, 2008). Two types of NHEJ are available to mammalian cells: the pathway that is dependent of DNA PKcs (referred to as D-

NHEJ) and the alternative (or “back-up”) pathway (referred to as B-NHEJ), which is also known as microhomology-mediated end-joining (MMEJ) (Ciccia and Elledge, 2010; West, 2003). Therefore, we will discuss known somatic DNA repair pathways and their potential role in spermatids when supported by published data.

4.3.1 Homologous recombination and single-strand annealing

Given the haploid character spermatids, HR could not take place as sister chromatids or homologous chromosomes are not available for recombination. Considering that HR precisely restores the genomic sequence of the broken DNA ends by utilizing sister chromatids as template for DNA repair, HR usually occurs in late S2 and G phase of the cycle in mammals (Kass and Jasin, 2010), whereas spermatids are considered to be in a G1-like phase (Ahmed et al., 2010). Upon resection at DNA breaks by the MRN complex, two different pathways are usually possible: HR or SSA (Wold, 1997). The SSA pathway could use repetitive DNA sequences to promote the DNA repair of DSBs in spermatids (Hartlerode and Scully, 2009; Motycka et al., 2004). This pathway is known to introduce errors such as deletions, insertions and even be a substrate for chromosomal translocations (Griffin and Thacker, 2004). There is currently no evidence that spermatids use SSA rather than NHEJ to repair DSBs, but some key proteins of this pathway, although also part of the NER pathway (see Table 1 and Table 2), are present during spermiogenesis including ERCC1 (Hsia et al., 2003; Paul et al., 2007) as well as XPF (Shannon, 1999).

DNA repair pathways	Implicated proteins	Typical error
Homologous recombination (HR)	BRCA1/2, Rad51, XRCC2, LIG1, RPA	Error free
Single strand annealing (SSA)	ERCC1, ERCC4 (XPF), Rad52	Indels (++) Chromosomal translocation (++)
Non-homologous end-joining DNA PK dependant (D-NHEJ)	DNA PKcs, Ku70, ARTEMIS, XRCC4, LIG4, XLF (NHEJ1)	Indels (+) Chromosomal translocation (+)
Alternative non-homologous end joining (B-NHEJ)	PARP, XRCC1, LIG3	Indels (+) Chromosomal translocation (+)

Table 2. DNA double-strand break repair pathways and their typical error. (+) Occasional, (++) frequent (Ciccia and Elledge, 2010; Griffin and Thacker, 2004).

4.3.2 Non-homologous end joining

Besides SSA, B-NHEJ and D-NHEJ are potentially available for the repair of double-strand breaks during spermiogenesis (Leduc et al., 2008a; Leduc et al., 2008b). In somatic cells, NHEJ pathways promote the religation of DSBs, introducing small insertions and deletions. NHEJ pathways operates throughout the cell cycle but are most active during G1 phase

because HR cannot proceed during that time (Daley et al., 2005). Spermatids provide a similar cellular context as G1 phase of somatic cells. However, dynamics of DNA repair by NHEJ pathways, as illustrated in irradiated round spermatids, are much slower (Ahmed et al., 2010). According to Ahmed and colleagues both pathways are present and active during mouse spermiogenesis: spermatids of SCID mice, lacking the D-NHEJ because of the absence of DNA PKcs, displayed slower repair than those from wild type mice (Ahmed et al., 2010). Further studies on the end-joining pathways in elongating spermatids will be required as these are known to be error-prone in somatic cells. This may also be the case in spermatids. Although an attenuation of the frequency of mutations may be found in the germ line (Walter et al., 1998), the chromatin remodeling in spermatids may still be the key differentiation steps where most of the new mutations repertoire is being produced for the transmission to the next generation.

## 5. Possible consequences on fertility and genetic integrity

### 5.1 Incomplete DNA repair

High level of sperm DNA fragmentation, sperm DNA damages and chromatin alterations decrease pregnancy rates in natural fertilization, intrauterine insemination and *in vitro* fertilization (Bungum et al., 2007; Duran, 2002; Evenson et al., 1999; Evenson and Wixon, 2006; Spano et al., 2000; Zini, 2011). Moreover, pregnancy loss following *in vitro* fertilization (IVF) or intracytoplasmic sperm injection (ICSI) treatments has also been attributed to poor sperm DNA integrity (Zini et al., 2008). Although sperm DNA fragmentation is more frequent in infertile men, sperm of fertile men display DNA fragmentation but to a lesser extent (Bellver et al., 2010; Brahem et al., 2011; Perrin et al., 2009; Rybar et al., 2009; Venkatesh et al., 2011; Watanabe et al., 2011). After fertilization, the oocyte can efficiently repair some paternal DNA damages (Brandriff and Pedersen, 1981; Marchetti et al., 2007), but in the case of highly damaged sperm DNA, this could exceed the DNA repair capacity of the oocyte leading to some genetic aberrations, developmental arrest or pregnancy loss.

### 5.2 Faulty repair

#### Structural aberrations

Chromosomal structural aberrations such as translocations, deletions and inversions, may originate from meiotic recombination involving non-allelic repeated DNA sequences (Heyer et al., 2010). However, since about 80% of chromosomal rearrangements are reported to be of paternal origin (Buwe et al., 2005; Thomas et al., 2006) and that male and female meiosis involves similar genetic mechanisms (Gu et al., 2008; Thomas et al., 2006), one can surmise that yet another process unique to male gametogenesis may be involved. We therefore hypothesize that the chromatin remodeling process in spermatids, generating transient double-strand breaks, may provide the proper context for faulty repair and induction of transgenerational polymorphism. In addition, it is tempting to speculate that, because chromatin condensation occurs, free DNA ends are brought in juxtaposition, increasing the chance of NHEJ repair involving two different chromosomes, which may lead to translocations. Interestingly, chromosomes possess their own territory within the nucleus of somatic cells and in sperm cells (Hazzouri et al., 2000; Manvelyan et al., 2008; Mudrak et al., 2005; Zalenskaya et al., 2000). Moreover, chromosomes known to have higher translocation rates have close chromosomal territories in somatic cells (Branco and Pombo, 2006; Brianna

Caddle et al., 2007; Manoj S Gandhi and Nikiforov, 2009). Thus, chromosomes with close chromosomal territories in spermatids could be more prone to interchromosomal translocation during chromatin remodeling. In addition, the potential for the spermatidal chromatin remodeling to produce non-B DNA structure may exacerbate the propensity for spermatids to produce translocation (Hidehito Inagaki and Kurahashi, 2009; Raghavan and Lieber, 2004). Further investigation is needed on the mechanism and potential involvement of chromatin remodeling in such events.

### **Insertions and deletions**

As outlined above, NHEJ repair pathways are known to create insertions and deletions (indels) as they use microhomology to join the two DNA ends. This type of mutations may be particularly harmful in coding sequences as it may cause codon frameshifts or alteration of mRNA splicing. Moreover, Y chromosomes microdeletions, associated with increased male infertility, may exhibit the classical signature of micro-homology based DNA repair pathways such as SSA and B-NHEJ as the deletion occurs between repetitive, often palindromic sequences (Paulo Navarro-Costa and Plancha; Yen, 1998). Although SSA is available during most of spermatogenesis, the B-NHEJ signature on the highly repetitive Y chromosome may be indicative of a faulty DNA repair in spermatids as this pathway is inhibited during meiosis.

### **Dynamic mutations**

Several diseases with dynamic mutation, characterized by the expansion over generation of a trinucleotidic repeat (TNRs), are associated with a paternal bias of expansion, such as Huntington disease (HD), spinocerebellar ataxia type 2 and 7 (Cancel et al., 1997; Stevanin et al., 1998; Zühlke et al., 1993). TNRs are microsatellites sequences in coding or non-coding region of the genome. Their stability, which is relative to the chance of adopting a secondary structure, is dependent of the nature of the sequence and the length of the TNR (Kovtun et al.; Tóth et al., 2000). The exact mechanism of TNR expansion or contraction is still not clear. However, studies show strong evidences that TNR expansion in the huntingtin gene occurs during spermiogenesis; in a transgenic mouse model carrying the mutated human gene, an increased length of the CAG repetition was observed in mature spermatozoa but not in early haploid spermatids and other tissues. Kovtun and McMurray also demonstrated the involvement of MSH2, a protein involved in the gap repair and mismatch repair pathways, as this expansion was absent in HD mice MSH2<sup>-/-</sup> (Albin and Tagle, 1995; Kovtun and McMurray, 2001). The remodeling chromatin of spermatids may promote secondary structure formation at TNRs, providing an ideal context for such mutations.

## **6. Conclusion**

The chromatin remodeling in spermatids involves transient DNA-strand breaks. Our group has generated evidences that a significant number of double-strand breaks are generated. These DSBs seemingly trigger a damage response as H2AFX is phosphorylated and a DNA repair pathway yet to be identified. As a result, no such DSBs are found in the mature sperm unless a pathological condition prevails. The non-templated DNA repair of these transient DSBs are expected to introduce small mutations likely distributed randomly across the haploid genome although their distribution remains to be established. Meiosis is well known to produce new combination of alleles but is not a primary driver of sequence divergence (Noor, 2008). Potential new gene function must arise through point mutations or

indels and the present review suggests chromatin remodeling of spermatids as an appropriate context for such induction of new polymorphism and possible translocations. Although the frequency of mutation may be lower in germ cells than in somatic cells (Walter et al., 1998), we hypothesize that most of the new mutations generated during spermatogenesis may be through the process of endogenous strand breaks and repair during spermiogenesis. Owing to the 1% chance for a random mutation to occur within genes due to exonic representation in the genome, most mutations are expected to be silent but, if within coding sequences, potential alteration of gene function may be transmitted. In summary, repair of the endogenous DSBs in spermatids may represent a new male-driven source of genetic variation.

## 7. References

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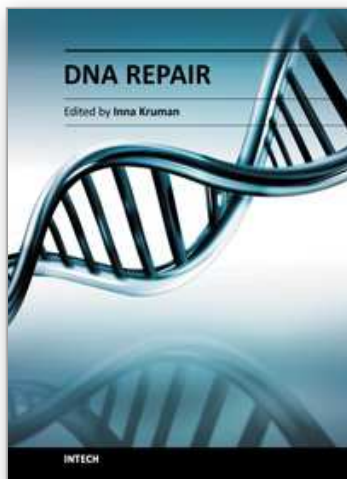
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Unit 405, Office Block, Hotel Equatorial Shanghai  
No.65, Yan An Road (West), Shanghai, 200040, China  
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元  
Phone: +86-21-62489820  
Fax: +86-21-62489821

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